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(54) Use of urea and thiourea compounds for elimination or detoxofication of superantigens from body fluids

(57) A material which has a highly selective absorption of super antigens contains urea bonds or thiourea bonds and remains active even in a high protein concentration solution in the neutral region. The material can be used in a body fluid purifying column for eliminating or detoxification of super antigens, or as a wound dressing material with super antigen adsorbing properties.

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Description

The present invention relates to a material for detoxification or elimination of super antigens such as staphylococcal enterotoxin and streptococcal exotoxin. This material binds with super antigens existing in a protein solution at a high concentration such as in the human blood etc., it is preferably used as an (antidotal) medicine for reducing or eliminating toxic activity of super antigens, as a purification column for eliminating the super antigen or as a wound dressing material.

Super antigens are a group of proteins which can directly bind with major histocompatibility antigen class II proteins (hereinbelow called "MHC class II" in some cases) on an antigen presenting cell without the necessity for passage, during processing, through the antigen presenting cell to be different from that for conventional antigens and furthermore, stimulate a T-cell by forming a complex with this MHC class II and T cell. Several restrictions exist in binding the T-cell for the conventional antigens, and the number of T-cells reacting with this is usually at most one per ten thousand, but as the super antigen binds only to the variable region of the β - chain of the T-cell receptor, certain kinds of super antigens stimulate one T-cell among five T-cells. As the result, it is thought that super antigens stimulate extraordinarily the immune system to generate fevers, rash and hypotension during sepsis and vomiting during food poison or autoimmune diseases (D.L.Murray et al, American Society of Microbiology News, (1995), 61(5), 229). As the super antigens, staphylococcal enterotoxin, Streptococcal exotoxin, Yersinial exotoxin, certain virus proteins and heat shock proteins have been confirmed and it is possible that other super antigens will be found in future.

Up to this time, as those substances which have affinity with these super antigens, antibodies to these super antigens (P.M.Rosten et al., Journal of Clinical Microbiology, (1987), 25(2), 327), major histocompatibility antigen class II proteins and a part thereof (J.K.Russell et al. Biochemical and Biophysical Research Communications, (1990), 169, 696)and ion exchange resins (H. Igarashi et al., Infection and Immunity, (1984), 44(1), 175) are known and they have been used as binding substances for adsorbing super antigens in blood and culture supernatant. However, most of these binding substances are proteins and peptides and they are easily deactivated by sterilization. In addition, affinity between ion exchange resins and super antigens is easily reduced by the influence of pH of the solution; specificity is decreased in the neutral region. Therefore, they are not suitable as a material with sufficient affinity for super antigens in solutions having a high protein concentration as blood, foods etc., where the pH should be kept neutral.

The present invention seeks to address the problem of solving the disadvantages of conventional technologies by providing a material with excellent selective affinity for super antigens even in a high protein concentration solution at a pH in the neutral region, with residual activity after sterilization and which is inexpensive. Namely, the material of the present invention has a high affinity for super antigens and it can bind with super antigens existing in body fluids such as blood and urine, foods, drinks and medicines. It is possible by such binding that the toxin-like activities of the super antigens can be eliminated (detoxification) by changing such properties of the super antigens as their conformations or by shielding the binding sites with MHC class II or/and T-cells. Namely, when the material of the present invention is used as a medicine, it is possible to effectively treat the effects of food poisoning, sepsis and autoimmune diseases or to prevent them from occurring. In addition, if this material is water-insoluble, it becomes possible by using this to eliminate super antigens from body fluids such as blood and urine, foods, drinks and medicines to to treat the effects of food poisoning, sepsis and autoimmune diseases and to prevent them from occurring. In particular, it is suitable as a body fluid purifying column for eliminating super antigens or as a super antigen-adsorbing wound dressing material.

In addition, it may be used as a quantitative measuring material and it is possible to diagnose food poisoning, sepsis and autoimmune diseases. The present invention provides a material which enables diagnosis and therapy of these diseases and prevents them from occurring.

We found that a material containing a urea bond or a thiourea bond has an affinity with staphylococcal enterotoxin and Streptococcal exotoxin. That is, a first aspect of the present invention provides a material for use in treatment of a human or animal body for eliminating or detoxifying super antigens wherein said material comprises a urea bond or a thiourea bond. A second aspect of the invention provides the use of such a material in the preparation of an agent employed in the treatment of a human or animal body for elimination or detoxification of super antigens, while a third aspect provides the use of such a material for in vitro elimination or detoxification of super antigens. The material preferably comprises a group capable of forming a hydrogen bond and an aromatic substituent. Thus, the material may be employed, for example, as a medicament, for example, on a wound dressing, or as a sorbing agent either during circulation of blood or plasma out of and thereafter back into a human or animal body, or during in vitro sorption procedures, which may be carried out on a body fluid such as blood, plasma or serum.

A fourth aspect of the present invention provides a urea or thiourea compound, useful as the material employed in performance of the first to third aspects of the invention, which compound has the following formula:

$$R^1-NHCNH-(R^3-NHCNH)_k-R^2$$

which compound contains (1) a group capable of forming a hydrogen bond and (2) an aromatic substituent, and

X is O or S;
k is 0 or a positive integer; and
each of R^1, R^2 and R^3, which may be the same as or different from one another, is any one of a group capable of forming a hydrogen bond and an aromatic substituent.

Where k ≥ 2, it is preferable that a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent alternate with one another.

As a group capable of forming a hydrogen bond, an amino group or a hydroxyl group, particularly, a secondary or tertiary amino group is preferable. More preferably, in such a compound,

(i) R^1 and/or R^3 contains a structure of formula (II)

$$-(CH_2)_nCHOH(CH_2)_m- \quad \text{(II); or}$$

(ii) at least one of R^1, R^2 and R^3 contains a structure of the formula (III)

$$-(CH_2)_n\underset{\underset{R^4}{|}}{N}(CH_2)_m- \quad \text{(III)},$$

wherein in each of formulae (II) and (II) each of m and n, independently of one another, is selected from 0 and 1-10.

A fifth aspect of the present invention provides a body fluid purifying column comprising the above mentioned material

A sixth aspect of the present invention provides a wound dressing material comprising the above mentioned material.

Embodiments of the invention will now be described in more detail.

There are no special limitations as the substituent of the urea bond or the thiourea bond and aliphatic compounds such as a hexyl group, octyl group and dodecyl group and alicyclic compounds such as cyclohexane and cyclopentane may be used, but aromatic compounds such as those containing a phenyl group, naphthyl group and anthranyl group are more preferably used. In addition, derivatives such as those containing an aminohexyl group, monomethylaminohexyl group, dimethylaminohexyl group, aminooctyl group, aminododecyl group and tolyl group, chlorophenyl group, nitrophenyl group, diphenylmethyl group and aminodiphenylmethyl group are also preferably used. Moreover, compounds containing, as substituents, a group capable of forming hydrogen bonds such as an amino group, hydroxyl group, carboxyl group and mercapto group are preferably used. For example, such compounds as those having a hydroxyl group, for example, hydroxypropane, 1,3 diamino-2-hydroxypropane, hydroxybutanone, hydroxybutyric acid and hydroxypyrimidine and glucides such as monosaccharides, oligosaccharides and polysaccharides such as glucose, glucosamine, galactosamine, maltose, cellobiose, sucrose, agarose, cellulose, chitin, chitosan and derivatives thereof, and those compounds having amino group, for example, diethylenetriamine, triethylenetetramine, tetraethylenpentamine, dipropylenetriamine, polyethyleneimine, N-methyl-2,2'-diaminodiethylamine are preferably used. The material of the present invention has most preferably both a group derived from an aromatic compound and a group capable of forming a hydrogen bond derived from a compound such as an amino group - or a hydroxyl group-containing compound (including glucides or their derivatives) as substituents on a urea bond or a thiourea bond.

In addition, any of monomers, oligomers or polymers can be employed as a material used in accordance with the present invention. Thus, compounds such as polymers derived wholly or in part from compounds having the above described substituents are also in the range of materials of the present invention. Namely, as polymers which partly or wholly comprise units having the above described substituents, those having a repeating unit of synthetic polymers such as nylon, polymethyl methacrylate, polysulfone, polystyrene, polyethylene, polyvinyl alcohol and polytetrafluoroethylene and natural polymers such as cellulose, collagen, chitin, chitosan and their derivatives, are preferably used. Namely, it is preferable to introduce urea bonds or thiourea bonds into these synthetic polymers prepared by homopolymerization, copolymerization or blending or into natural polymers. In addition, those products prepared by coating an inorganic material such as metals, ceramics and glass with an appropriate polymer are also preferably used.

In addition, polyurea or polythiourea wherein a plurality of urea bonds or thiourea bonds exist in the molecular structure is preferable as a material for use in the present invention. In this case, any one of the above described substituents can be used as the substituent of the urea bond or the thiourea bond, it is most preferable to incorporate both an aromatic compound and a compound having a group capable of forming a hydrogen bond such as amino group or hydroxyl group-containing compound (including glucides and their derivatives).

The material of the present invention can be synthesized by generally known methods. For example, when a urea bond or a thiourea bond is introduced into an aliphatic compound and an aromatic compound, a method wherein an isocyanate derivative or an isothiocyanate derivative is reacted with an amino compound can be used. As the isocyanates or the isothiocyanates, for example, aliphatic isocyanates or isothiocyanates such as ethyl isocyanate, stearyl isocyanate, n-butyl isocyanate, iso-butyl isocyanate, n-propyl isocyanate, methyl isothiocyanate, ethyl isothiocyanate, n-butyl isothiocyanate, benzyl isothiocyanate, hexamethylenediisocyanate, cyclohexyl isocyanate, cyclohexylisothiocyanate and cyclohexyldiisocyanate can be used, but aromatic isocyanates or isothiocyanates such as phenyl isocyanate, chlorophenyl isocyanate, fluorophenyl isocyanate, bromophenyl isocyanate, nitrophenyl isocyanate, tolylphenyl isocyanate, methoxyphenyl isocyanate, 1-naphthyl isocyanate, 4,4'-diphenylmethanediisocyanate, 3,3',5,5'-tetraethyl-4,4'-diisocyanatediphenylmethane, phenyl isothiocyanate, chlorophenyl isothiocyanate, fluorophenyl isothiocyanate, nitrophenyl isothiocyanate, tolyl isothiocyanate, methoxyphenyl isothiocyanate and 1-naphthyl isothiocyanate, are more preferably used. In addition, as amino group of the amino compounds used in the present invention, any of primary amino group, secondary amino group or tertiary amino group can be used and as amino compound, for example, any one of sec-octyl-amine, 6-amino-n-caproic acid, 3-amino-1-propene, α -amino-isobutyric acid, aminopyridine, aminobenzenesulfonic acid, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, dipropylenetriamine, N-methyldiaminodiethylamine, polyethyleneimine etc., can be used; however taking the reactivity of the amono group into consideration, it is preferable to have a primary amino group at at least one reaction site. In addition, amino compounds with a hydroxyl group can be more preferably used. Namely, aliphatic amines such as 2-ethanolamine, 3-propanolamine, 6-hexanolamine, 1,3-diamino-2-hydroxypropane and glucamine and derivatives of N-methyl-1,3-diaminopropanol or aromatic amines such as 4-aminophenol, diaminophenol aminohydroxypyrimidine, diaminohydroxypyrimidine and diamonohydroxypyrazole or amino acids such as serine and tyrosine can be used. In addition, it is preferred that an amino compound with a hydroxyl group is synthesized from a compound only with a hydroxyl group or a compound only with an amino group by reacting it with epichlorohydrin and an amino compound or 1,3-dibromo-2-hydroxypropane etc. In this case, the mixing ratio of the amino compound to an isocyanate derivative or an isothiocyanate derivative can be arbitrarily selected and it is preferable that the amount of amino group is an equimolecular quantity or excess to the amount of isocyanate group to suppress the reaction of the hydroxyl group with isocyanate or isothiocyanate group. In addition, when urea bonds or thiourea bonds are introduced into a glucide, the same method as described above can be used. Namely, when a glucide with an amino group such as chitosan or glucosamine is used, the above described isocyanate derivative or isothiocyanate derivative can be reacted. In the case of a glucide that has no amino group such as cellulose, after the hydroxyl group of the glucide is activated using epichlorohydrin or trisilylchloride, an amino group is introduced by reacting it with ammonia or diaminoethane and urea bonds or thiourea bonds can be introduced into a glucide utilizing this amino group.

In addition, when the material of the present invention is an oligomer or a polymer, for example, a method wherein an oligomer or a polymer with an isocyanate group, a carboxyl group or an active ester group of a carboxylic acid such as succinimide group is reacted with the amino group of a urea derivative or a thiourea derivative, is preferably used. As the amino group used in the reaction, because of the low reactivity of the terminal amino group of the urea bond or the thiourea bond, it is preferable to use an amino group existing on another position. In addition, a method wherein an oligomer and a polymer each with an amino group or an oligomer and a polymer wherein an amino group is introduced by using ammonia, diamenoethane, 1,3-diaminopropane or 1,3-diamino-2-hydroxypropane are reacted with an isocyanate derivative or an isothiocyanate derivative, is preferable. Functional groups such as amino groups, isocyanate groups, carboxyl groups or an active ester group of a carboxylic acid such as a succinimide group can be introduced if necessary, into an oligomer and a polymer.

In addition, when the material of the present invention is polyurea or polythiourea, for example, a method wherein a polyisocyanate derivative or a polyisothiocyanate derivative is reacted with a polyamino compound can be used. Ordinarily, as the amount of the reagent, 0.1-5 mole polyamine is preferably used to 1 mole polyisocyanate or polyisothiocyanate. As the polyisocyanate or polyisothiocyanate, hexamethylenediisocyanate, cyclohexyldiisocyanate, tolylene diisocyanate, 4,4'-diphenylmethanediisocyanate, 3,3',5,5'tetraethyl-4,4'-diisocyanate-diphenylmethane, xylylene diisocyanate, methylene-bis(4-phenyl isothiocyanate) etc., are preferably used. In addition, as the polyamino compound, diaminoethane, diaminopropane, 1,3-diamino-2-hydroxypropane, N-methyl-1,3-diamino-2-propanol, diamino-phenol, N,N'-diaminopiperazine, diethylenetriamine, triethylenetetramine, tetraethylenpentamine, polyethyleneimine, dipropylenetriamin , N-methyldiaminoethylamin etc., are preferably used.

All the above described reactions are performed as the standard at a reaction temperature of 0-150°C and for a reaction time of 0.1-24 hours. In addition, even though the reaction solvent is not always necessary, but the reaction is ordinarily performed in the presence of a solvent. As the solvents which can be used, aliphatic hydrocarbons such as

methanol, ethanol, isopropyl alcohol, n-butanol, hexane, acetone, N,N-dimethylformamide and dimethyl sulfoxide, aromatic hydrocarbons such as benzene, toluene and xylene, halogenated hydrocarbons such as dichloromethane, chloroform and chlorobenzene, ethers such as diethyl ether, tetrahydrofuran and dioxane are cited. The product can be purified by column chromatography and recrystallization after the reaction liquid is treated by such an ordinary after-treatment as filtration and concentration. In addition, in the case of a water-insoluble material, washing it by using a glass filter is also a preferable method.

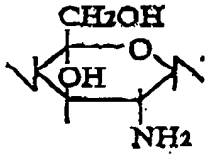
Water-insoluble materials of the present invention are preferably used, for example, for a super antigen elimination column, a wound dressing material or a quantitative measuring material. There is no special limitation on their shapes and when they are used as an elimination column, shapes such as beads, fibers, hollow fibers, yarns, nets, braids, woven or knitted fabrics of coarse structure (randomly packed, spirally wound or packed with fragments thereof) are preferable and when they are used for a quantitative measuring material, shapes such as beads, plates, yarns, nets, braids, woven or knitted fabrics of coarse structure (randomly packed, spirally wound or packed with fragments thereof) are preferable and in the case of wound dressing materials, shapes such as fabrics and films are preferable. As materials having urea bonds, porous chitosan beads, "CHITOPEARL BCW-3001" and "CHITOPEARL BCW-3501" (Fuji Spinning Co., Ltd) are commercially available. However, these chitosan beads have been used as carriers for enzyme immobilization and are not yet known to have an affinity for super antigens. On the other hand, polyether urethane urea has urea bond and has been used as a material for medical use; however, it does not have affinity for super antigens.

Especially preferred embodiments will now be described in more detail with reference to the accompanying drawings and the following Examples. In the drawings,

Figure 1 shows an infrared spectrum of chitosan beads modified with p-chlorophenyl isocyanate;
Figure 2 shows the results of adsorption tests on super antigens by means of circulation method; and
Figure 3 shows an infrared spectrum of a polyurea derivative.

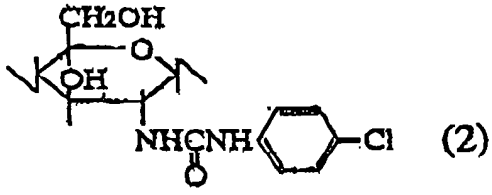
Example 1 Introduction of urea bonds into chitosan beads and a super antigen adsorption test using the beads

Beads of chitosan ("CHITOPEARL AL-01" manufactured by Fuji Spinning Co., Ltd.) with a structural formula (1), having a sedimentary volume of 12 ml, a dry weight of 1.0g and a particle diameter of 0.1 mm were stirred in 20 ml N,N-dimethyl-formamide for five minutes. Then the beads and the solution were separated by means of a glass filter



(1)

This operation which needed 5 minutes per operation was repeated 20 times to substitute N,N-dimethylformamide for the water content. These beads were gradually added into 100 ml N,N-dimethylformamide in which 1 g p-chlorophenyl isocyanate was dissolved and the mixture was reacted for 1 hour at room temperature while it was stirred. Thereafter, the beads and the solution were separated using a glass filter and washing was performed by stirring these beads in 20 ml N,N-dimethylformamide for 5 minutes. This washing operation was repeated 20 times to eliminate completely unreacted p-chlorophenyl isocyanate. Then, a washing operation with distilled water was performed in the same way to substitute distilled water for N,N-dimethylformamide and chitosan beads with a structural formula (2) were obtained. An infrared spectrum of the modified chitosan beads is shown in Figure 1



(2)

By using these modified chitosan beads (2) and unmodified chitosan beads (1) as a control, adsorption of four kinds of super antigen, namely, staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C (SEC) and toxic shock syndrome toxin-1 (TSST-1) from a rabbit plasma were performed. The initial concentrations of these super antigens were 1 ng/ml and 1 ml of the above described chitosan beads after being autoclaved under high pressure at 120°C for 20 minutes was added into 10 ml plasma and the mixture was shaken at 37°C

for 60 minutes. The concentrations of four kinds of super antigen in the rabbit plasmas after reaction for 60 minutes were measured by means of an enzyme immun assay and the results wer shown in Table 1. As shown by this result super antigen adsorbability was provided to the chitosan beads by introducing urea bonds.

Table 1

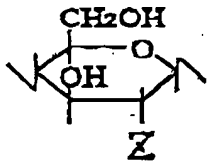
Super antigen adsorption tests for four kinds of super antigen in rabbit plasma samples using modified chitosan beads				
	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
Modified chitosan	519	393	453	288
Unmodified chitosan	1163	1120	960	939

Example 2 A super antigen adsorption test using a modified chitosan bead-circulation method

An adsorption test by means of a circulation method for a super antigen was performed using the unmodified chitosan beads (1) and the modified chitosan beads (2) of Example 1. 1 ml of the above described beads was introduced into a column and 10 ml rabbit plasma in which 1 ng/ml super antigen (TSST-1) was incorporated were circulated at 37°C for 60 minutes. The concentrations in the rabbit plasma after 5, 15, 30, 45 and 60 minutes were measured by means of an enzyme immune assay and the results were shown in Figure 2. Super antigen adsorbability under flow conditions similar to extracorporeal circulation were provided to the chitosan beads by introducing urea bonds in this manner.

Example 3 Super antigen adsorption tests using seven kinds of chitosan bead wherein urea bonds or thiourea bond were introduced

Phenyl isocyanate, p-tolyl isocyanate, 1-naphthyl isocyanate, phenyl isothiocyanate and p-chlorophenyl isothiocyanate were respectively reacted with chitosan beads by the same method as in Example 1, in addition, 4,4'-diphenylmethanediisocyanate and hexamethylenediisocyanate were reacted with chitosan beads by the same method as in Example 1 and then, terminal isocyanate groups were hydrolyzed by reacting them with distilled water for 12 hours at room temperature. Thereafter, the beads were washed thoroughly with distilled water. Modified chitosan beads each with a respective structural formula (3)-(9) were obtained by the above described method. Formulae (8) and (9) correspond to "CHITOPEARL BCW-3501" and "CHITOPEARL BCW-3501", respectively.



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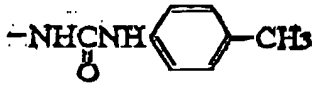

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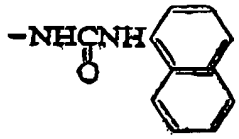

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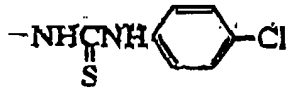


(7)

(8)

(9)

By using these seven kinds of modified chitosan bead and unmodified chitosan beads as a control, adsorption of super antigen (TSST-1) from rabbit plasma was performed in the same way as in Example 1. The initial concentration of TSST-1 was 1 ng/ml and 1 ml of the above described chitosan beads was added into 10 ml plasma, and the mixture was shaken at 37°C for 60 minutes and the concentrations of TSST-1 in the rabbit plasma samples after reaction were measured by means of an enzyme immune assay. The concentrations of TSST-1 after 60 minutes are shown in Table 2.

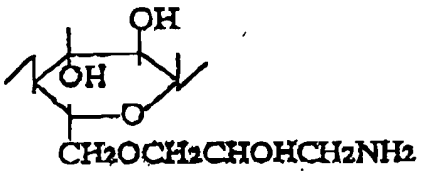
Table 2

Adsorption tests of TSST-1 from samples of rabbit plasma using seven kinds of modified chitosan bead	
Structural formula	TSST-1 concentration (pg/ml)
1	923
3	335
4	415
5	343
6	632
7	290
8	292
9	807

The structural formula (1) was for unmodified chitosan beads. As these results showed, super antigen adsorbability was provided to the chitosan beads by introducing urea bonds or thiourea bonds.

Example 4 Introduction of urea bonds into cellulose beads and a super antigen adsorption test using the beads

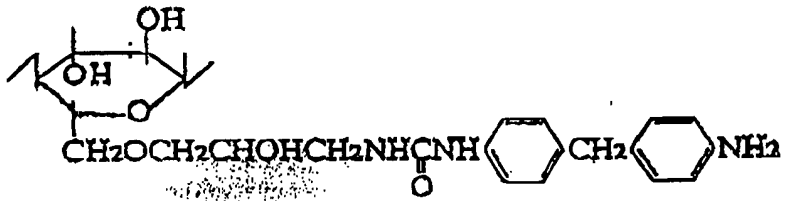
Aminated cellulose beads ("Amino-Cellulofine" manufactured by Chisso Co., Ltd., Tokyo Japan) with a structural formula (10), having a sedimentary volume of 12 ml and a particle diameter of about 0.2 mm were stirred in 20 ml N,N-dimethylformamide for five minutes. Then the beads and the solution were separated by means of a glass filter



(10)

This operation was repeated 20 times to substitute completely N,N-dimethylformamide for water content.

These beads were gradually added into 100 ml N,N-dimethylformamide in which 0.1 g 4,4'-diphenylmethanediisocyanate was dissolved and the mixture was reacted for 1 hour at room temperature while it was stirred. Thereafter, the beads and the solution were separated using a glass filter and washing was performed by stirring these beads in 20 ml N,N-dimethylformamide for 5 minutes. This washing operation was repeated 20 times to eliminate completely unreacted 4,4'-diphenylmethanediisocyanate. Then, it was reacted with the distilled water at room temperature for 12 hours and the terminal isocyanate groups were hydrolyzed to prepare amino groups. Thereafter, by washing thoroughly the beads with distilled water cellulose beads were obtained with a structural formula (11).



(11)

By using these modified cellulose beads (11) and unmodified cellulose beads (10) as a control, adsorption of a super antigen (TSST-1) from rabbit plasma was performed in the same way as in Example 1. The initial concentration of TSST-1 was 1 ng/ml and 1 ml of the above described cellulose beads was added into 10 ml plasma and the mixture was shaked at 37°C for 60 minutes. The concentration of TSST-1 after the reaction was measured by an enzyme immune assay and the concentration of TSST-1 after 60 minutes are shown in Table 3.

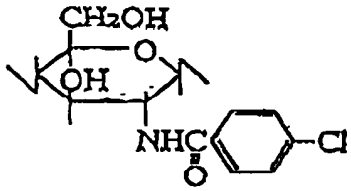
Table 3

TSST-1 adsorption from samples of rabbit plasma using modified cellulose beads	
	Concentration of TSST-1 (pg/ml)
Modified cellulose	485
Unmodified cellulose	948

As shown by this result, super antigen adsorbability was provided to the cellulose beads by introducing urea bonds.

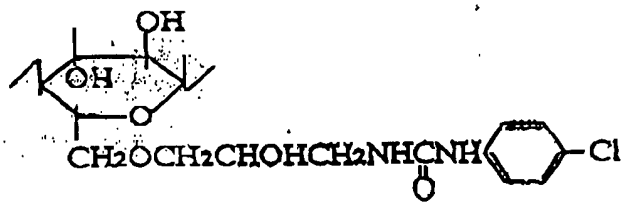
Example 5 (Comparative Example 1) Comparative tests on adsorption functions of TSST-1 between beads with amide bonds and urethane bonds and beads with urea bonds.

Chitosan beads with amide bonds (structural formula (12)) were prepared by reacting chitosan beads ("CHITOPEARL AL-1" with a structural formula (1)) with p-chlorobenzoyl chloride. This was a product wherein urea bonds of chitosan beads with the structural formula (2) prepared in Example 1 were replaced with amide bonds.

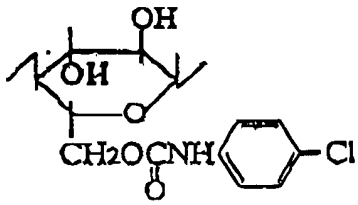


(12)

In addition, by the same method as that of Example 1, cellulose beads with urea bonds of structural formula (13) were prepared by reacting the aminated cellulose beads ("Aminocellulofine") used in Example 4 with p-chlorophenyl isocyanate. On the other hand, cellulose beads wherein urethane bonds were introduced (structural formula (14)) were prepared by reacting cellulose beads ("Cellulofine GCL2000") with p-chlorophenyl isocyanate in the presence of triethylamine for 12 hours.



(13)



(14)

By using these beads, adsorption of super antigen (TSST-1) from rabbit plasma was performed. The initial concentration of TSST-1 was 1 ng/ml and 1 ml of the above described beads was added into 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. The concentrations of TSST-1 in the samples of rabbit plasma after reaction were measured by an enzyme immune assay and the results after 60 minutes are shown in Table 4.

Table 4

Comparative tests on adsorption functions of TSST-1 between beads with amide bonds or urethane bonds and beads with urea bonds (in rabbit plasma)		
Structural formula	Bonding mode	Concentration of TSST-1 (pg/ml)
(2)	Urea bond	310
(12)	Amide bond	947
(13)	Urea bond	398
(14)	Urethane bond	972

As shown by this result, super antigen adsorbability was not provided by introducing amide bonds and urethane bonds and only in the case of urea bonds was super antigen adsorbability provided.

Example 6 Confirmation of super antigen specificity

By using modified chitosan beads (the structural formula (2)) prepared in Example 1, adsorbability to TSST-1 as a super antigen was investigated and adsorbabilities to bovine serum albumin (BSA) and human immunoglobulin G (IgG) as non-super antigens were investigated. Each protein was dissolved in rabbit plasma so as to obtain the concentration of 1 ng/ml. 1 ml of the above described beads was added into 10 ml of the plasma, the mixture was shaken at 37°C for 60 minutes and the concentration of the protein in the plasma after reaction was measured by the enzyme immuno assay. The concentrations of the protein after 60 minutes are shown in Table 5. As these results showed, super antigen adsorbability was provided by introducing urea bonds but there was no adsorbability to other proteins while high specificity to super antigens was exhibited.

Table 5

Adsorption characteristics of various proteins using modified chitosan beads	
	Concentration of protein
TSST-1	345 pg/ml
Bovine serum albumin	946 pg/ml
Immunoglobulin G	946 pg/ml

Example 7 Preparation of polyurea derivatives

0.32 g 1,3-diamino-2-hydroxypropane (hereinafter abbreviated as DAHP) was dissolved in 40 ml dimethyl sulfoxide (hereinafter abbreviated as DMSO). 10 ml DMSO solution in which 0.63 g 4,4'-diphenylmethanediisocyanate (hereinafter abbreviated as MDI) was dissolved were dropped into this solution while it was stirred. After the whole amount of 10 ml had been added, reaction was performed at 25°C for one hour. Thereafter, 60 ml distilled water were added into the reaction liquid while it was stirred. A white precipitate which formed here was recovered by centrifugal separation and the recovered precipitate was washed five times with 50 ml methanol. Then, the precipitate was dried under vacuum to obtain 0.88 g polyurea derivative (hereunder abbreviated as DAHP polyurea). An infrared spectrum of the polyurea derivative is shown in Figure 3. As shown in Figure 3, the existence of hydroxyl group and urea bond were confirmed. Similarly, another polyurea derivative (hereunder abbreviated as DAP poyurea) was obtained using 1,3-diaminopropane (hereunder abbreviated as DAP) instead of DAHP.

Example 8

Adsorption tests of super antigens using polyurea derivatives prepared in Example 7 were performed by the same method as that in Example 1. For use as a control, a polyurethane derivative was prepared in the same way as Example 7 except that 1,3-propanediol was used instead of DAHP, triethylamine was included in the reaction mixture and the reaction was performed for 12 hours.

The initial concentrations of SEA, SEB, SEC and TSST-1 were 1 ng/ml and each 1 ml of DAHP polyurea, DAP polyurea, polyurethane was added in 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. All of DAHP polyurea, DAP polyurea and polyurethane were used after high pressure steam sterilization at 121°C for 20 minutes. The concentrations of four kinds of super antigen in rabbit plasma after 60 minutes reaction were measured by enzyme immuno assay and the results are shown in Table 6. As these results showed, although polyurethane does not adsorb super antigens, it became clear that polyurea adsorbs super antigens and by introducing hydroxy groups to the polyurea, the adsorbability was improved.

Table 6

Adsorption tests of four kinds of super antigen in rabbit plasma using polyurea				
	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
DAHP polyurea	820	357	338	435
DAP polyurea	728	810	735	749
polyurethane	925	880	956	890

Example 9 Preparation of polystyrene fiber with a hydroxyl group-containing urea derivative on its side chain

Islands-in-a-sea type composite fiber described in U.S. Patent No. 4,661,260 (thickness: 2.6 denier; number of the islands:16) comprising of 50 wt parts of sea component (mixture of 46 wt parts of polystyrene and 4 wt parts of polypropylene) and 50 wt parts of islands component (polypropylene) were reacted in a mixed solution of 50g of N-methylol-α-chloracetamide, 400g of nitrobenzene, 400g of 95% sulfuric acid and 0.85g of paraformaldehyde at 20°C for one hour. Then the fiber was washed with nitrobenzene, and thrown into water to stop the reaction. After that, the fiber was washed again with warm water. Thus, chloroacetoamidemethylated crosslinked polystyrene fiber (hereinafter abbreviated as AMPSt fiber) was obtained.

10 g DAHP were dissolved in 500 ml DMSO. 20 g AMPSt fiber (corresponding to 20 mmol chloro content) were added into this solution while it was stirred. The reaction was performed at 25°C for 6 hours. Thereafter, AMPSt fiber was washed on a glass filter with 500 ml DMSO and then, successively with 50 ml N,N-dimethylformamide. After washing, 1 g each of AMPSt fiber was added into 50 ml DMF wherein one of the below described isocyanates or isothiocyanates was dissolved.

Table 7

Isocyanates or isothiocyanates used for reaction with polystyrene fiber	
Reaction product	Isocyanates or isothiocyanates used for reaction
(a)	0.23 g phenyl isocyanate
(b)	0.30 g para-chlorophenyl isocyanate
(c)	0.30 g meta-chlorophenyl isocyanate
(d)	0.30 g ortho-chlorophenyl isocyanate
(e)	0.27 g para-fluorophenyl isocyanate
(f)	0.32 g para-methoxyphenyl isocyanate
(g)	0.26 g para-tolyl isocyanate
(h)	0.32 g para-nitrophenyl isocyanate
(i)	0.33 g 1-naphthyl isocyanate
(j)	0.48 g 4,4'-diphenylmethanediisocyanate
(k)	0.70 g 3,3',5,5'-tetraethyl-4,4'-diisocyanate-diphenylmethane
(l)	0.24 g cyclohexyl isocyanate
(m)	0.33 g hexamethylenediisocyanate
(n)	0.19 g n-butyl isocyanate
(o)	0.26 g phenyl isothiocyanate
(p)	0.33 g para-chlorophenyl isothiocyanate
(q)	0.27 g cyclohexyl isothiocyanate
(r)	0.22 g n-butyl isothiocyanate

The reaction was performed at 25°C for one hour. Thereafter, the reaction product was washed on a glass filter with 200 ml DMSO and 500 ml distilled water. The compounds obtained from the reaction with each isocyanate or isothiocyanate were referred to respectively as (a)-(r).

In addition, a sample of AMPSt fiber wherein a p-nitrophenyl group was introduced by reacting p-nitrophenyl isocyanate was added into 100 ml aqueous sodium hydrosulfite solution (0.1 g/ml) and it was converted into a p-aminophenyl group by reduction at 60°C for 4 hours; it was referred to as the compound (s)

Example 10 Adsorption of super antigens using polystyrene fibers with urea derivatives on their side chains

Adsorption tests of super antigens using modified polystyrene fibers prepared in Example 9 were performed by the same method as that in Example 1. The initial concentrations of SEA, SEB, SEC and TSST-1 were 1 ng/ml and each 1 g of the modified AMPSt fiber was added in 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. The modified AMPSt fibers were used after high pressure steam sterilization at 120°C for 20 minutes. The concentrations of four kinds of super antigen in rabbit plasma after 60 minutes reaction were measured by enzyme immuno assay and the results are shown in Table 8.

As a control, an AMPSt fiber (t) wherein amide bonds were introduced instead of urea bonds by reacting benzoyl chloride instead of isocyanate under the same condition was used. In addition to that, an AMPSt fiber (u) wherein phenyl isocyanate was reacted after DAP was reacted instead of DAHP was also evaluated.

As these results showed, it became clear that as the polystyrene fiber (t) where there was no urea bond exhibited no adsorbability of super antigens; adsorbability of super antigens was exhibited by introducing urea bonds. In addition, it was shown that higher adsorbability of super antigens was exhibited by modifying with an aromatic isocyanate than with an aliphatic isocyanate. In addition, the adsorbability of sup r antigens was reinforced by introducing hydroxyl groups.

Table 8

Adsorption of super antigens using polystyrene fibers each with a urea derivative on their side chains

Modified AMPSt	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
(a)	450	382	360	462
(b)	330	382	385	399
(c)	332	381	362	352
(d)	450	462	475	488
(e)	400	412	388	426
(f)	723	733	584	659
(g)	448	285	383	433
(h)	621	589	589	603
(i)	425	335	385	418
(j)	352	350	330	320
(k)	768	812	750	796
(l)	766	801	789	732
(m)	682	762	787	698
(n)	702	785	788	776
(o)	483	955	354	477
(p)	336	975	358	401
(q)	770	798	774	762
(r)	777	774	793	802
(s)	822	852	885	956
(t)	975	985	1022	1006
(u)	802	798	822	785

Example 11 Preparation of polystyrene fiber with an amino group-containing urea derivative on its side chain

0.8 g triethylenetetramine was dissolved in 500 ml DMSO. 1.0 g AMPSt fiber (which corresponded to 2 mmol chloro content) were added into this solution while it was stirred. The reaction was performed at 25°C for 12 hours. Thereafter, AMPSt fiber was washed on a glass filter with 500 ml DMSO and then, successively with 50 ml N,N-dimethylformamide. After washing, the AMPSt fiber was added into 50 ml DMF in which 0.30 g p-chlorophenyl isocyanate had been dissolved. Thereafter, AMPSt fiber was washed on a glass filter with 200 ml DMSO and then successively with 200 ml distilled water and AMPSt fiber (v) was obtained.

Example 12 Adsorption of super antigens using polystyrene fibers with an amino group-containing urea derivatives on their side chains

Using the AMPSt fiber (v) obtained by Example 11, adsorption of super antigens were performed in the same way as in Example 1. As a control, AMPSt fiber (t) used in Example 10 was used. The initial concentrations of SEA, SEB, SEC and TSST-1 were 1 ng/ml and 1 g of the above described AMPSt (v) fiber were incorporated into 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. Each AMPSt (v) fiber was used after being sterilized under high pressure steam at 121°C for 20 minutes. The concentrations of four kinds of super antigen in the rabbit plasmas after reaction for 60 minutes were measured by an enzyme immun assay and the results are shown in Table 9. As shown by this result, it is clear that an amino group-containing urea derivative has super antigen adsorbability.

Table 9

Adsorption of super antigens using polystyrene fibers with an amino group-containing urea derivative on their side chains				
Modified AMPSt	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
(V)	241	287	302	197
(T)	956	1001	981	975

As illustrated above, material containing urea bonds or thiourea bonds with excellent selective binding characteristics with super antigens even in a high protein concentration solution in the neutral region and remaining activity even after sterilization and being inexpensive is provided by embodiments of the present invention. By using the material in accordance with the present invention, it is possible that the activities of super antigens existing in body fluids such as blood and urine, foods, drinks and medicines can be removed (detoxified), it is possible to treat food poisoning, sepsis and autoimmune diseases and to prevent them from occurring. In addition, as it is possible using water-insoluble materials to eliminate efficiently super antigens from body fluids such as blood and urine, foods, drinks and medicines, by a super antigen eliminating column and a wound dressing material, it is possible to treat food poisoning, sepsis and autoimmune diseases and to prevent them from occurring. In addition, as the material of the present invention can be used as a measuring material, it is possible to diagnose food poisoning sepsis and autoimmune diseases.

Claims

1. A material for use in treatment of a human or animal body for elimination or detoxification of super antigens characterised in that the material has therein a urea bond or thiourea bond.

2. A material for use according to claim 1, which material additionally has therein an aromatic ring.

3. A material for use according to claim 1 or 2, which material has therein a group which is capable of forming a hydrogen bond.

4. A material for use according to claim 3, which material is a compound of formula (I)

$$R^1-\overset{\overset{X}{\|}}{NHCNH}-(R^3-\overset{\overset{X}{\|}}{NHCNH})_k-R^2 \qquad (I)$$

which said compound has therein a group capable of forming a hydrogen bond and an aromatic substituent and,

X is O or S;
k is 0 or a positive integer; and
each of R^1, R^2 and R^3 is any one of a group capable of forming a hydrogen bond and an aromatic substituent.

5. A material for use according to claim 4, wherein, in the material, a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent are alternately repeated.

6. A material for use according to claim 3, 4 or 5, wherein the group which is capable of forming hydrogen bond is an amino group.

7. A material for use according to claim 6, wherein the amino group is a secondary or tertiary amino group.

8. A material for use according to claim 3, 4 or 5, wherein the group which is capable of forming a hydrogen bond is a hydroxyl group.

9. A material for use according to claim 8, wherein the hydroxyl group is a hydroxyl group of a glucide.

10. A material for use according to claim 9, wherein the glucide is at least one of chitosan, cellulose and derivatives of chitosan and cellulose.

11. A material for use according to any one of claims 2, 4 and 5, wherein, in the material, the aromatic substituent is a phenyl group, a naphthyl group or a derivative thereof at least one of the hydrogen atoms of which is substituted by F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 or CH_2PhNH_2.

12. A material for use according to claim 4 or 5, wherein, in the material, R^1 and/or R^2 contain(s) a structure of formula (II)

$$-(CH_2)_nCHOH(CH_2)_m- \qquad (II)$$

where n and m are each independently selected from 0 and 1-10.

13. A material for use according to claim 4 or 5, wherein, in the material, at least one of R^1, R^2 and R^3 contains a structure of formula (III)

$$-(CH_2)_n\overset{R^4}{\underset{|}{N}}(CH_2)_m- \qquad (III)$$

where R^4 is hydrogen or a C_{1-10} alkyl group and n and m are each independently selected from 0 and 1-10.

14. A material for use according to claim 4 or 5, wherein, in the material, k is 1 to 200.

15. A material for use according to any preceding claim, which material comprises a substrate.

16. A material for use according to claim 15, wherein the substrate comprises at least one of polystyrene, polysulfone, polymethyl methacrylate and derivatives of any of these.

17. A material for use according to claim 15 or 16, wherein the substrate is a fiber.

18. A material for use according to claim 17, wherein the fiber is an islands-in-a-sea type fiber.

19. A material for use according to any preceding claim, which material is water-insoluble.

20. Use, in the preparation of an agent employed in the treatment of a human or animal body for elimination or detoxification of super antigens, of a material as defined in any preceding claim.

21. Use, for elimination or detoxification of super antigens, of a material as defined in any one of claims 1 to 19.

22. A body fluid purifying column comprising a material as defined in any one of claims 1 to 19.

23. A wound dressing material comprising a material as defined in any one of claims 1 to 19.

24. A compound of formula (I)

$$R^1-NHC(=X)NH-(R^3-NHC(=X)NH)_k-R^2 \qquad (I)$$

which said compound has therein a group capable of forming a hydrogen bond and an aromatic substituent and,

X is O or S;
k is 0 or a positive integer; and
each of R^1, R^2 and R^3 is any one of a group capable of forming a hydrogen bond and an aromatic substituent.

25. A compound according to claim 24, a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent are alternately repeated.

26. A compound according to claim 24 or 25, wherein the group capable of forming a hydrogen bond is an amino group.

27. A compound according to claim 26, wherein the amino group is secondary or tertiary amino group.

28. A compound according to claim 24 or 26, wherein the group capable of forming a hydrogen bond is a hydroxyl group.

29. A compound according to claim 28, wherein the hydroxyl group is a hydroxyl group of a glucide or a derivative thereof.

30. A compound according to any one of claims 24 to 26, wherein the aromatic substituent is a phenyl group, a naphthyl group or a derivative thereof at least one of the hydrogen atoms of which is substituted by F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 or CH_2PhNH_2

31. A compound according to any one of claims 24 to 30, wherein R^1 and/or R^3 contain(s)a structure of formula (II)

$$-(CH_2)_nCHOH(CH_2)_m- \qquad (II)$$

where n and m are each independently selected from 0 and 1-10.

32. A compound according to any one of claims 24 to 30, wherein at least one of R^1, R^2 and R^3 contains a structure of formula (III)

$$-(CH_2)_n\overset{R^4}{\underset{|}{N}}(CH_2)_m- \qquad (III)$$

where R^4 is hydrogen or a C_{1-10} alkyl group and n and m are each independently selected from 0 and 1-10.

33. A compound according to any one of claims 24 to 26, wherein k is 1 to 200.

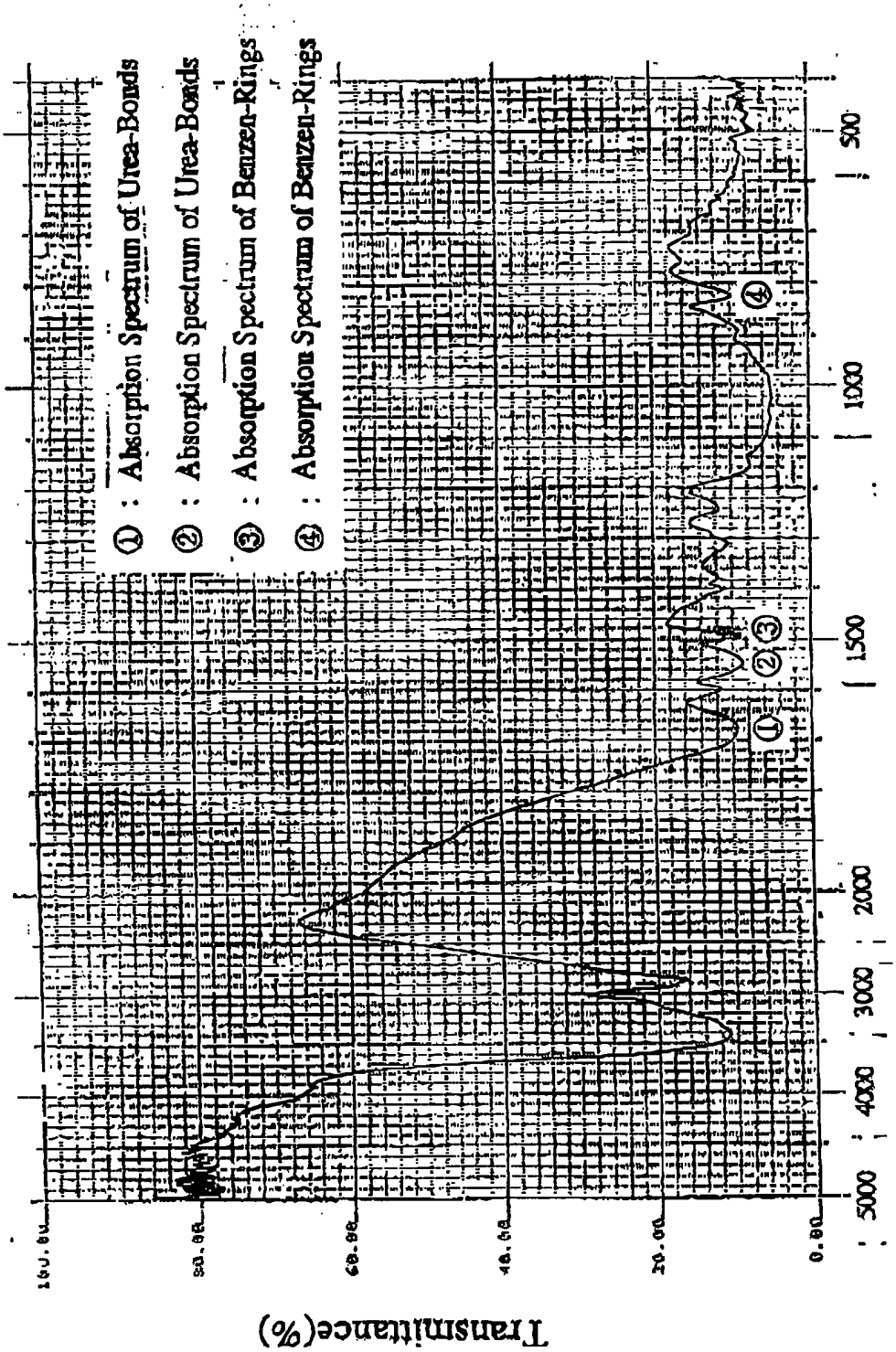
34. A method of removing a super antigen from a fluid by passing the super antigen-containing fluid through a column filled with a material for elimination or detoxification of the super antigen, characterised in that the material has therein a urea bond or thiourea bond.

35. A method according to claim 34, wherein the fluid is blood, plasma or serum

36. A method of removing a super antigen from blood or plasma, comprising the successive steps of:

(a) contacting the blood or plasma with a material capable of elimination or detoxification of the super antigen, which material has therein a urea bond or thiourea bond; and
(b) separating the blood or plasma from the material.

Fig. 1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② : Absorption Spectrum of Urea-Bonds
③ : Absorption Spectrum of Benzen-Rings
④ : Absorption Spectrum of Benzen-Rings
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Wave Number (cm⁻¹)
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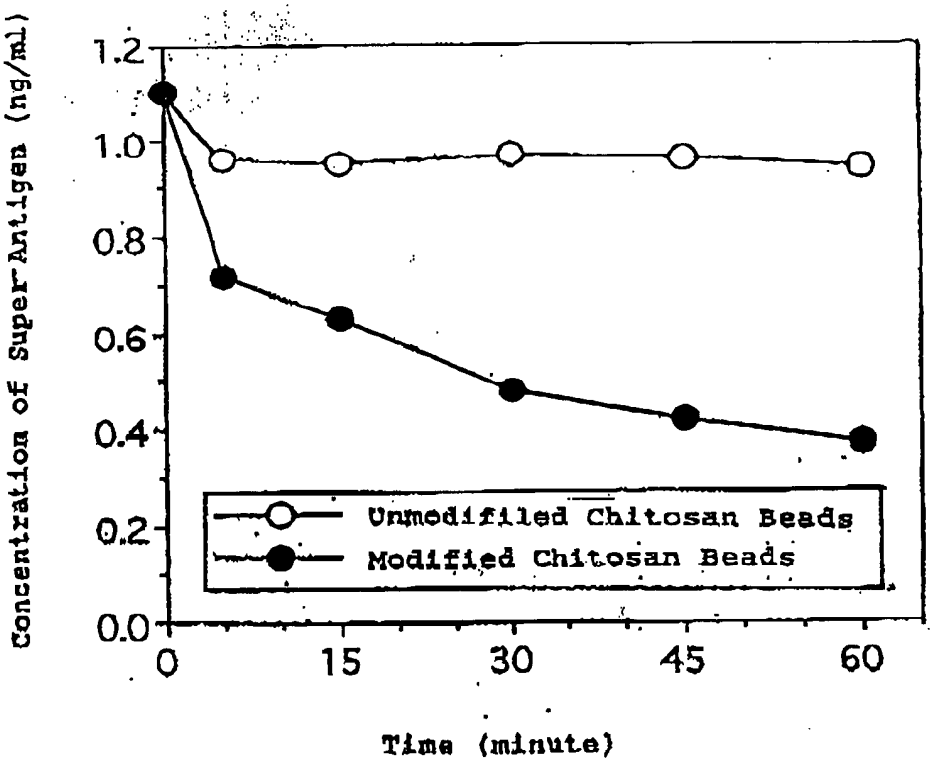
2000
1500
1000
500
0.00
20.00
40.00
60.00
80.00
100.00

Fig. 2


Concentration of SuperAntigen (ng/ml)
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1.0
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0.6
0.4
0.2
0.0
0
15
30
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Time (minute)
Unmodified Chitosan Beads
Modified Chitosan Beads

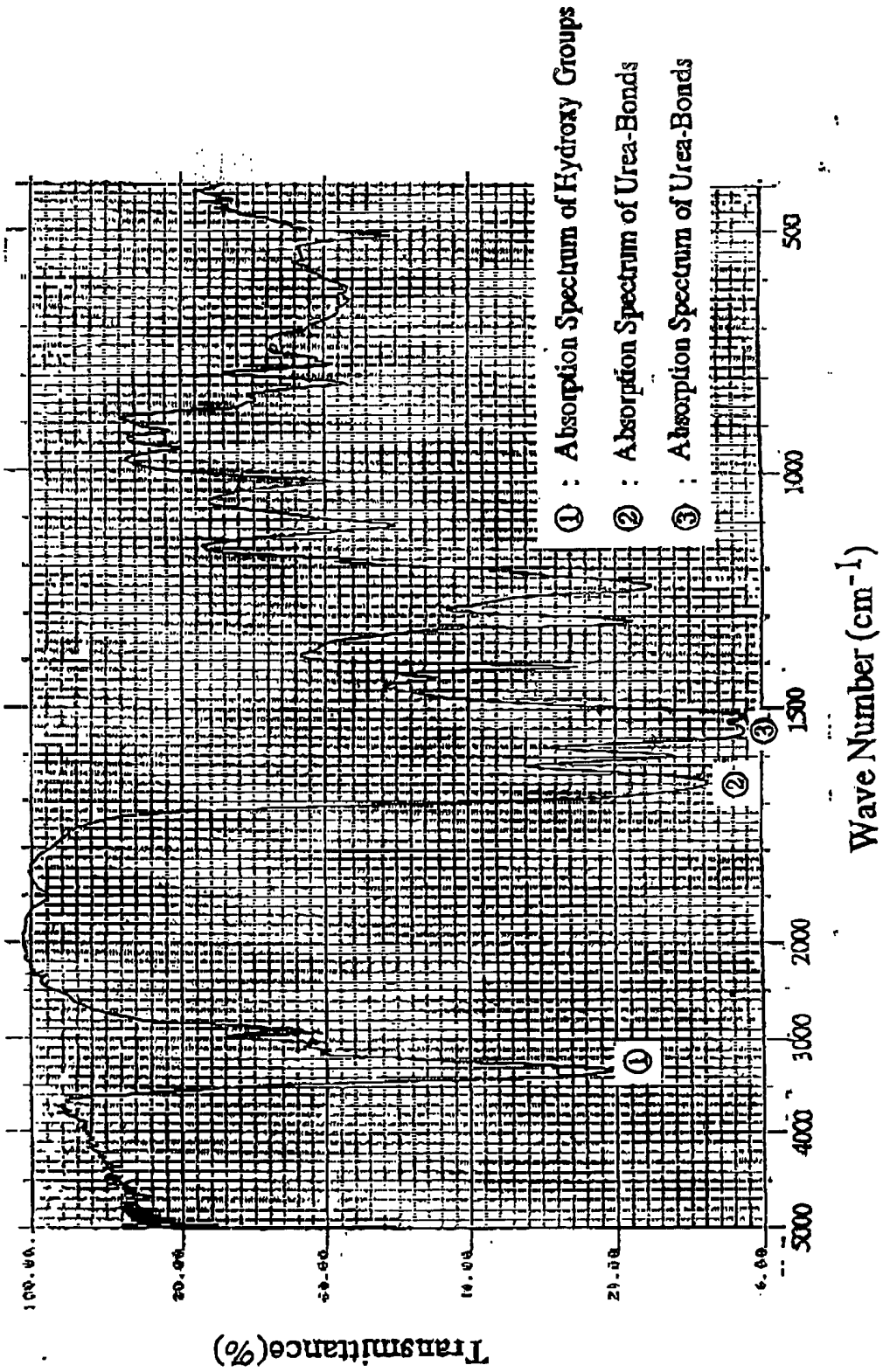
① : Absorption Spectrum of Hydroxy Groups
② : Absorption Spectrum of Urea-Bonds
③ : Absorption Spectrum of Urea-Bonds
Transmittance(%)
Wave Number (cm⁻¹)
5000
4000
3000
2000
1500
1000
500

Fig.3